MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

Previous studies from this laboratory have shown that stimulation of the spinal cord with more than one electrode could produce hindlimb flexion/extension or bladder contractions of large magnitude at reduced current density when compared to a single electrode. The responses to focal microstimulation with two or more (maximum of four) electrodes often produced additive responses. In some instances, greater than additive or synergistic responses were seen. In our initial studies of hindlimb flexion/extension, we used four electrodes with a spacing of 0.5 mm together with a variety of stimulus presentations including simultaneous stimulation as well as various amounts of interleaving of the stimulus on two or more electrodes. An abstract summarizing some of these studies has been submitted to the Society for Neuroscience. A copy of that abstract is attached to this progress report. These types of studies continued during this quarter. In addition, during this quarter we examined wider spacing of electrodes including 1.0 mm separation of each of four electrodes. Testing of stimulation with two electrodes was performed at separations varying from 0.5 mm to 3.0 mm. Stimuli were applied to the microelectrode in a simultaneous or an interleaved mode. A variety of responses were seen. With electrodes at 1.5 mm or less and synchronized stimuli, additive or synergistic responses were produced, while interleaving usually reduced the responses. At electrode separations of 2 to 3 mm with synchronized stimuli additive and synergistic responses were elicited, with interleaving stimuli producing a larger response than synchronized (simultaneous) stimulation.

During this quarter studies on the correlation between flexion/extension EMG and flexion/extension torque also continued. An abstract summarizing some of our most recent studies was submitted to the 20th Annual International Conference of the IEEE-EMBS. A copy of that abstract is included with this progress report.

II. Hindlimb Extension Torque about the Knee Joint Elicited by Multiple Electrode Stimulation of the L_6 Spinal Cord.

The purpose of these experiments was to examine the use of two or more (maximum of four) microelectrodes to produce extension of the lower hindlimb (shank) about the knee joint to stimulation of several sites in the L_6 spinal cord. Of particular interest was: (1) the optimal distance separating the electrodes, (2) whether simultaneous or interleaved patterns of stimulation were more effective in eliciting extension torque of the shank, and (3) whether additive, synergistic, or inhibitory responses resulted from the activation of pairs of electrodes at different distances of separation.

Our standard preparation was used in these studies. Cats were anesthetized with pentobarbital (25-35 mg/kg I.V.). A rotational torque sensor recorded flexion or extension torque from the shank. Fine wire electrodes positioned in several hindlimb muscles recorded EMG from shank extensors and flexors. The stimulating electrodes were an array of four activated iridium electrodes, each with an exposed tip surface area of 300 μm^2. The four electrodes were separated by 0.5 mm (center-to-center) in one group of experiments and by 1.0 mm (center-to-center) in another set of experiments. The electrodes of the array were fixed in their relative position to each, such that the array moved through the spinal cord as a unit with their tips at the same approximate level. Individual electrodes could not be positioned independently. Stimuli could be applied to one or any combination of the four electrodes. When two or more electrodes were used for microstimulation, the stimuli could be delivered simultaneously or in various interleaved modes. In all experiments summarized in this progress report, the four electrodes were oriented in the rostrocaudal direction approximately parallel to the "motoneuron pools." The most rostral electrode is designated as "A" and the most caudal as

"D" with "B" and "C" in the middle (see Figure 1A for a schematic diagram of the electrode positions).

Electrode separation proved to be an interesting parameter in terms of pattern of stimulation. With two electrodes separated by 2.0 mm or less the largest responses were elicited with simultaneous activation of both electrodes (Figures 1B, 2B, and 2C). Interleaving the stimuli (i.e., delaying the stimulus to the second electrode by a specific amount) always produced a reduction in the torque response. This can be seen in Figures 1B, 2B, and 2C where the electrode separation is 0.5, 1.0, and 2.0 mm respectively. During simultaneous stimulation of both electrodes (0.0 msec interleave time) the response is larger than with any amount of interleaving for separations of 2 mm or less. The largest responses were elicited with simultaneous stimulation at 0.5 and 1.0 mm separation (Figures 1B and 2C), and a decrease in response is seen with interleaving at all interleave times. At a 3.0 mm electrode separation, however, the response is enhanced with interleaving (Figure 2A). The enhanced torque response is seen at all interleave times and is also reflected in the EMG recorded (not shown in Figures). This data suggests that the interactions between electrodes are different when the electrodes are close together compared to electrodes which are further apart. In addition, as electrodes are separated by larger distances interleaving of stimuli may be a more effective means of activating "motoneuron pools" than simultaneous stimulation. The exact underlying neurophysiological mechanism of these differences is unknown. These types of studies will be continued into the next quarter with additional studies on electrode separation and stimulus patterns.

During this quarter we also examined the additive and synergistic effects of simultaneous stimulation of pairs of electrodes, at separation of 0.5 mm to a maximum of 3.0 mm. At any given depth the wide separation of electrodes often positions one or two electrodes at sites which

produce little or no torque response to individual electrode stimulation. At these sites a variety of responses could be generated from very large synergism to inhibition. Figure 3 shows a variety of responses that can be produced from pairs of electrodes deep (3.8 mm) in the ventral horn. Two of the electrodes (A and C) produced quite larger responses to increasing intensities of stimulation while the other two electrodes (B and D) produced only small responses even at high intensities (100 μA) of microstimulation. By examining pairs of electrodes at various distances apart a variety of interesting torque responses were recorded which had not been seen in experiments with electrodes positioned at less than 1.5 mm separation. For example, Figure 3E shows a small inhibitory response between two electrodes separated by 3.0 mm, one electrode (D) elicited almost no response while the other electrode produced a large response at all intensities. However, if electrode D, which produces almost no response, is paired with electrode C (1.0 mm apart) a dramatic synergistic response is produced (Figure 3F). A third very interesting variation is the response produced by the two electrodes (B and D) which elicited almost no torque individually (Figure 3D). Activated simultaneously they produced a very dramatic increase in torque. The total torque produced (6.0 Ncm) is not large (notice the y-axis), but these sites have the ability to facilitate each other as well as more effective sites to enhance the torque response and reduce the current density. This data would suggest that certain sites in the ventral horn are facilitory to hindlimb extension while producing very little response when stimulated alone. Likewise, some areas of the ventral horn may be inhibitory to motor responses. Some of these sites may, when stimulated in certain combination, produce significantly enhanced responses at acceptable current densities. These and similar studies using a variety of stimulus patterns will be continued during the next quarter.

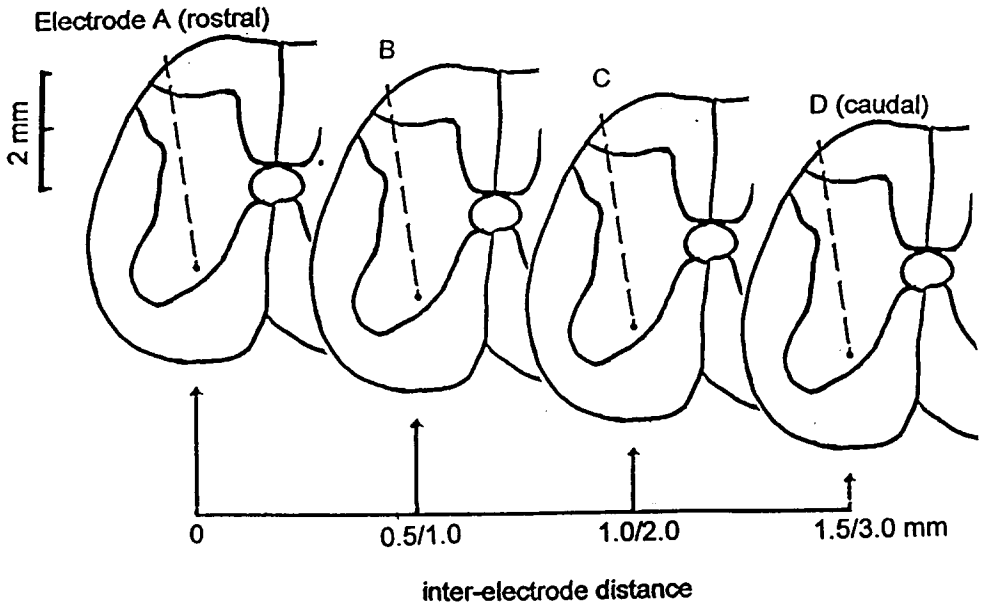
Figure 1A: Schematic diagram showing the typical pathways a four-electrode array would take passing from the dorsal surface of the L_6 spinal cord to the ventral funiculus. The inter-electrode distances are shown for 0.5 mm and 1 mm separations. Electrode A is the most rostral and D the most caudal electrode and B and C in between. This designation of the electrodes relative positions will be used in subsequent Figures and discussion in this progress report.

Figure 1B: Bar graphs showing the normalized extension torque responses to stimulation of the L_6 cord at different interleave times. Electrodes A and B are 0.5 mm apart. Zero (0) interleave is simultaneous stimulus of both electrodes. At an interleave of 1.5 msec the stimulus to electrode B (assuming A is stimulated first) is delayed by 1.5 msec. The larger the interleave number the longer the stimulus to the second electrode is delayed. At 12.5 msec interleave the stimulus to the second electrode (electrode B) is at the midpoint between the second stimulus pulse to A (at a stimulus frequency of 40 Hz there are 25 msec between pulses to A. 12.5 is half of 25 msec). Stimulus parameters: 75 μA, 0.2 msec pulsewidth, 40 Hz.

Figure 2: Bar graph showing the normalized extension torque responses to stimulation of the L_6 spinal cord at different interleave times. (Similar to Figure 1B.) Zero (0) interleave time is simultaneous stimulation of back electrodes. Each bar graph plot represents the responses of two electrodes at different distances of separation A=3 mm separation, B=2 mm separation and C=1 mm separation (see also Figure 1A). Interleave time is the amount of delay that the stimulus is present to the second electrode. Notice that the electrodes that are closer together produce a reduced response to interleaving (C and D also Figure 1B). However, at 3 mm separation, by interleaving the stimulus, the response becomes greater. Stimulus parameters are: 60-80 μA, 0.2 msec pulsewidth at 40 Hz.

Figure 3: Line graph showing the changes in extension torque to increasing intensity of microstimulation of the L_6 spinal cord. Each graph (A to F) shows response of two individual electrodes and the response to stimulation of both electrodes simultaneously. The electrodes are spaced 1 mm apart as depicted in Figure 1A. In this particular experiment, electrodes B and D had only very small responses while electrodes A and C had quite large responses. Notice that the Y-axis changes for each graph. Notice also that a variety of responses are shown. In D, neither electrode produces much of a response individually but when combined produces a synergistic response although total torque is not large (approximately 6.0 Ncm). In E, a small inhibitory response is elicited by electrodes A and D. In C, both additive and synergistic responses are shown depending on intensity of stimulation. In F, a large synergistic response is elicited when paired with an electrode that produced little or no response alone. Stimulus parameters are: 5-100 μA, 40 Hz, 0.2 msec pulsewidth, 12 seconds on 120 seconds off. Electrode depths are 4.2 mm.

A


Electrode A (rostral)
B
C
D (caudal)
2 mm
0
0.5/1.0
1.0/2.0
1.5/3.0 mm
inter-electrode distance

B

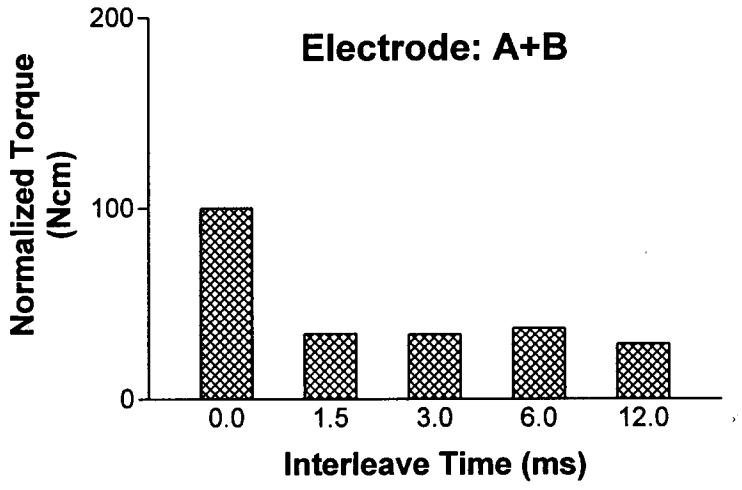

Electrode: A+B
Normalized Torque (Ncm)
200
100
0
0.0
1.5
3.0
6.0
12.0
Interleave Time (ms)

Figure 1

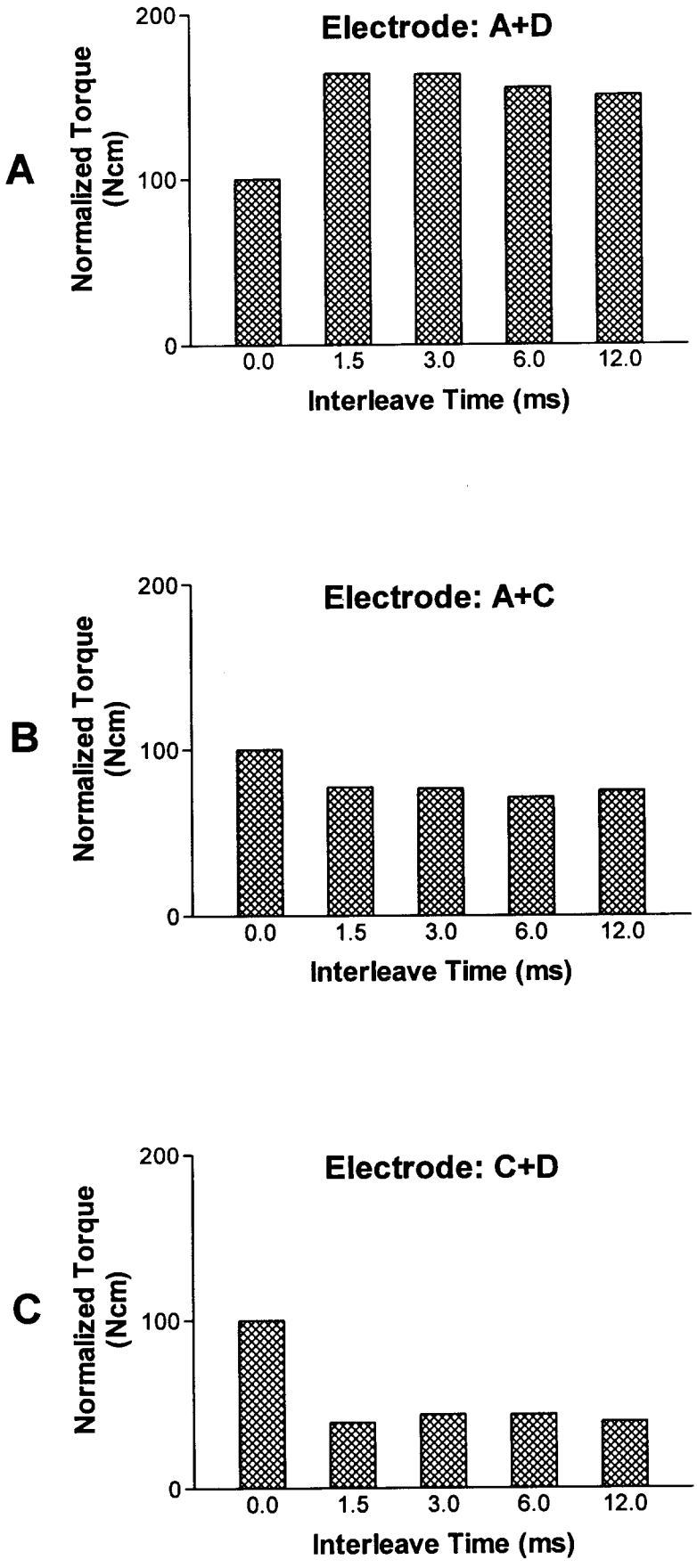
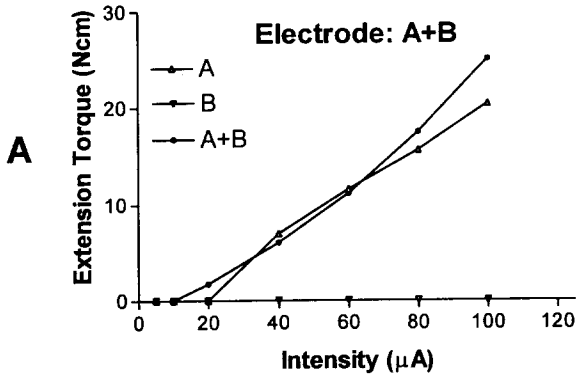
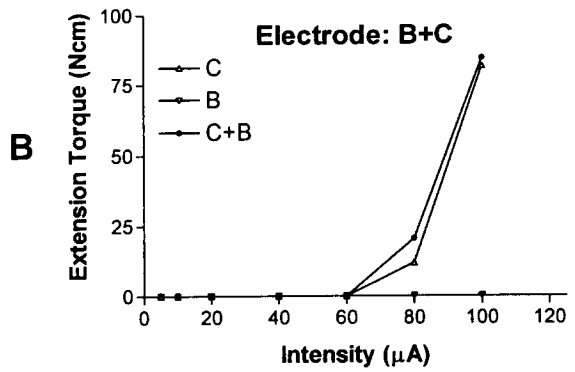
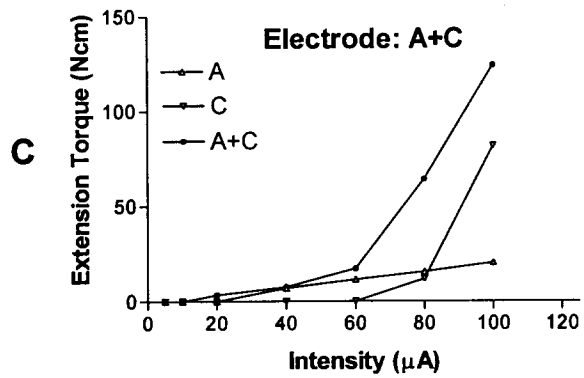
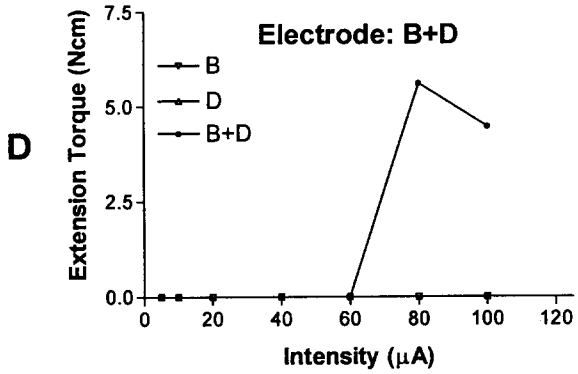

A
Electrode: A+D
Normalized Torque (Ncm)
200
100
0
0.0
1.5
3.0
6.0
12.0
Interleave Time (ms)
B
Electrode: A+C
Normalized Torque (Ncm)
200
100
0
0.0
1.5
3.0
6.0
12.0
Interleave Time (ms)
C
Electrode: C+D
Normalized Torque (Ncm)
200
100
0
0.0
1.5
3.0
6.0
12.0
Interleave Time (ms)

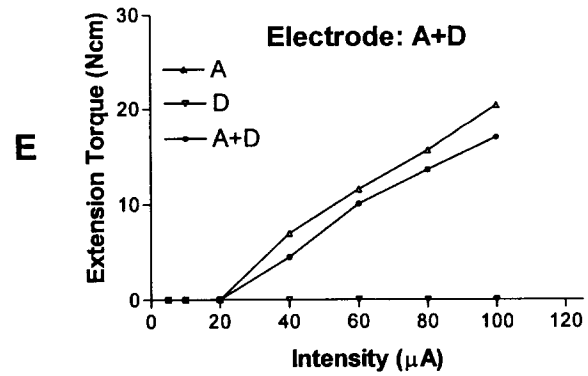
Figure 2


A
Electrode: A+B
A
B
A+B
Extension Torque (Ncm)
Intensity (μA)
0
10
20
30
0
20
40
60
80
100
120


B
Electrode: B+C
C
B
C+B
Extension Torque (Ncm)
Intensity (μA)
0
25
50
75
100
0
20
40
60
80
100
120


C
Electrode: A+C
A
C
A+C
Extension Torque (Ncm)
Intensity (μA)
0
50
100
150
0
20
40
60
80
100
120


D
Electrode: B+D
B
D
B+D
Extension Torque (Ncm)
Intensity (μA)
0.0
2.5
5.0
7.5
0
20
40
60
80
100
120


E
Electrode: A+D
A
D
A+D
Extension Torque (Ncm)
Intensity (μA)
0
10
20
30
0
20
40
60
80
100
120

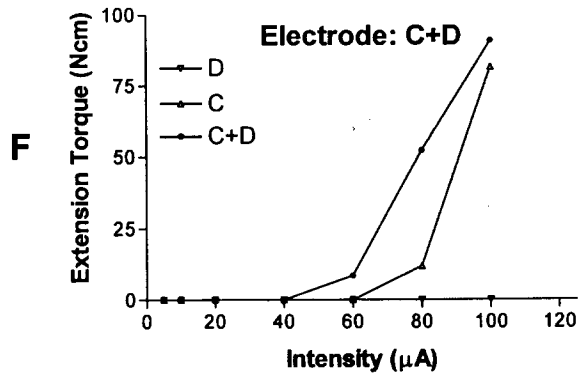

F
Electrode: C+D
D
C
C+D
Extension Torque (Ncm)
Intensity (μA)
0
25
50
75
100
0
20
40
60
80
100
120

Figure 3

The 20th Annual International Conference of the IEEE-EMBS

EMG ACTIVITY AND KNEE JOINT TORQUE EVOKED BY MICROSTIMULATION OF THE CAT L6 SPINAL CORD

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Abstract - The knee extensor and flexor EMG activity evoked by microstimulation of the cat L6 spinal cord were recorded while simultaneously monitoring the knee joint extension and flexion torques. Single fine-tipped (200 to 400 μm^2 surface area) activated iridium microelectrode was implanted in the left side of the L6 spinal cord. Large extension torque was produced by microstimulation in the ventral horn and small flexion torque was produced by microstimulation in the dorsal horn. The extensor EMG was much larger than the flexor EMG when large extension torque was produced. The flexor EMG was just slightly larger than the extensor EMG and both were small when small flexion torque was produced. When extension torque was generated, the extensor EMG increased rapidly with increasing stimulus intensity while the flexor EMG remained at almost the same level. These results showed that the L6 spinal cord of the cat is more effective in generating extension torque than flexion torque and that hindlimb flexor and extensor EMG is correlated with torque. Microstimulation of the spinal cord is a potential method to restore the low limb function for patients with spinal cord injuries.

I. INTRODUCTION

Functional neuromuscular stimulation (FNS) is a potentially useful technique to restore lower limb movement in individuals with spinal cord injuries. Considerable research has been done [1] in the past decade in order to find a useful FNS method to restore locomotor function for paraplegic patients, but success is limited. Most of the previous research focused on direct muscle or peripheral nerve stimulation. The major disadvantage of muscle stimulation is that limb movement and muscle contraction can alter the position of stimulating electrode within the muscle, and thus alter the potential field induced by the stimulus current [2]. This disadvantage makes it difficult to control limb movement by direct muscle even when closed-loop feedback control is employed [3]. In addition, fatigue is also a serious problem because large motor units with lower threshold and lower fatigue resistance are activated with every effective stimulus pulse. Conventional peripheral nerve stimulation methods excite larger nerve fibers first and then smaller ones in a "reverse recruitment" order [4]; further, a non-physiological synchronous firing of multiple nerve fibers is produced [5]. These two factors result in poor force gradation and rapid muscle fatigue. Finally, peripheral nerve stimulation presents difficulties in activating different groups of muscles independently [6].

Microstimulation in the lumbosacral spinal cord might offer several advantages over stimulation at more peripheral sites. Because neurons controlling different groups of muscles are located in different regions (motor-pools) of the spinal cord [7], electrical stimulation using a microelectrode might permit selective activation of small groups of neurons or axons, and thereby allow sequential and/or graded stimulation of inputs to the same muscle group.

In this paper, we studied the activation of knee extensor and flexor during microstimulation of the cat L6 spinal cord by measuring the muscle EMG activities and the joint isometric torque. The results showed that the L6 spinal cord of the cat is more effective in generating extension torque than flexion torque and that the EMG recorded from extensor and flexor muscle correlated well with the torque generated.

II. METHODS

Five male cats (3.7 kg to 5.3 kg) were studied while under pentobarbital anesthesia (20 to 25 mg/kg I.V.). The trachea was cannulated to maintain a patent airway and blood pressure was monitored by a catheter in the carotid artery. Core temperature was maintained between 35°C and 37°C using a heating pad. The spinal cord was exposed from L4 to S2 via a dorsal laminectomy. The dura mater was opened and each lumbosacral segment was identified. The skin, cut midsagittally, was tied to a frame along each margin to form a pool which was filled with warm oil or Krebs solution. All protocols in this study were approved by the animal care and

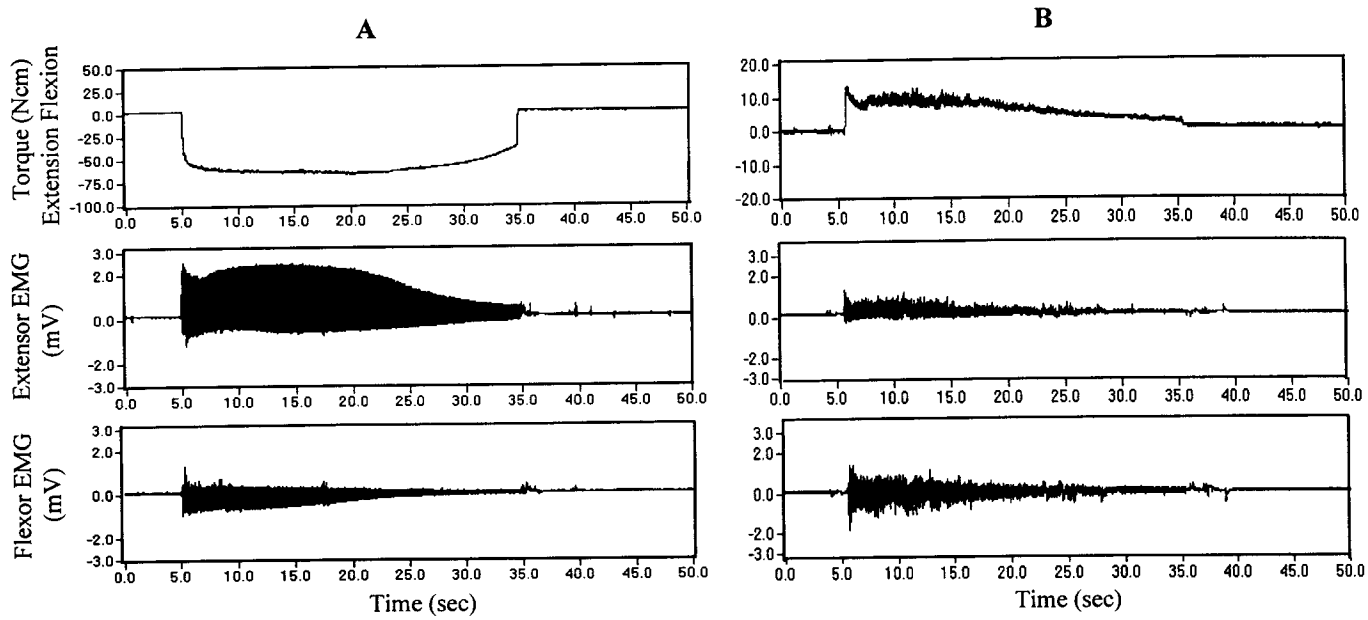

Fig. 1: Responses of isometric torques and EMGs produced by microstimulation of L6 spinal cord. Stimulation: 100 μA, 0.2 ms, 40 Hz. A: 5.0 mm depth; B: 0.4 mm depth.

use committee of University of Pittsburgh School of Medicine.

An aluminum bar was attached parallel to the left tibia by two screws. A rotational torque sensor (Eaton-Lebow 2120-500) was fixed to the aluminum bar with the sensor shaft aligned with knee joint axis. The joint angle was fixed at 120°. Flexion was measured as a positive isometric torque while extension as a negative torque. Two pairs of stainless steel wire electrodes (0.25 mm diameter) were implanted in the middle of knee joint extensors (quadriceps) and flexors (biceps femoris) respectively to record the EMG activities. The resultant torque and EMG signals were captured via National Instrument AT-MIO-16DE-10 A/D board in a PC (Dimension XPS P90C) running LabVIEW®3.1 for Windows. A stimulus duty cycle of 30 sec on, 120 sec off was used. Fifty seconds of data, along with 5 seconds of pretriggered data, were collected at 2000 samples/sec.

The left side of the L6 spinal cord segment was probed with a single fine-tipped (200 to 400 μm^2 surface area) activated iridium microelectrode which was advanced from the dorsal surface of the spinal cord in 200 μm increments. At each incremental stop, a train (40 Hz frequency, 30 sec duration) of constant-current, biphasic pulses (0.2 ms pulsewidth, 100 μA intensity) was delivered to the spinal cord. The microelectrode penetration was always started at the center of the dorsal root entry zone (DREZ), then the microelectrode was withdrawn and moved 200 to 400 μm medial/lateral and/or rostral/caudal to an adjacent location, and the testing repeated. Successive penetrations were made as long as the animal's physiological condition permitted (usually 12 to 24 hours). A single pulse (pulsewidth 0.2 ms) was also employed at depth of 4.0 mm from the surface of the L6 spinal cord. The extensor and flexor EMGs evoked by a single pulse were recorded for different stimulus intensities (10 μA to 150 μA). The magnitude of isometric torque was represented by the mean torque generated during the first 12 sec of microstimulation, since fatigue usually begins to occur after 12 sec to 15 sec stimulation. To evaluate co-activation of extensor and flexor muscles, the peak value of the extensor and flexor EMGs evoked by a single pulse stimulus and the integration of the first 12 sec of rectified EMGs were taken as representative of the muscle activation level.

III. RESULTS

Typical knee joint isometric torque responses and extensor/flexor EMGs produced by microstimulation of L6 spinal cord are shown in Fig. 1. The flexion torque evoked in the dorsal horn is much smaller than the extension torque evoked in the ventral horn of the L6 spinal cord (Fig. 1). Muscle fatigue was evident by a decreased isometric torque and decreased extensor/flexor EMGs. When a large knee extension torque was generated by microstimulation deep in the L6 spinal cord, the extensor EMG was much larger than the flexor EMG (see Fig. 1A). When a relatively small flexion torque was generated by microstimulation in the dorsal part of the L6 spinal cord, the flexor EMG was just slightly larger than the extensor EMG (see Fig. 1B).

The levels of EMG activity evoked by a single pulse stimulus (0.2 ms pulsewidth) at a depth of 4.0 mm are shown in Fig. 2,

where at the same location a 70 Ncm extension torque was produced by microstimulation at 100 μA intensity, 40 Hz frequency and 0.2 ms pulsewidth. As shown in Fig.2, the amplitudes of extensor EMG were always larger than those of flexor EMG at this particular site. The extensor EMG increased rapidly with increasing stimulus intensity, but the flexor EMG did not.

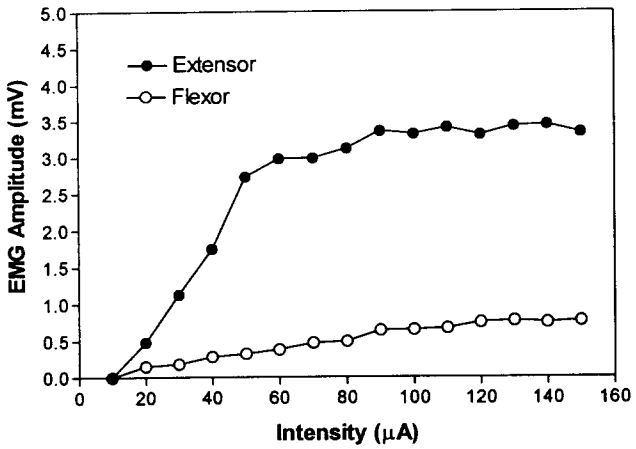

Fig. 2: Amplitudes of extensor and flexor EMGs evoked by a single pulse (0.2 ms) at 4.0 mm depth from the surface of the L6 spinal cord.

Fig. 3 shows the relation between the extensor and flexor EMGs and the isometric extension and flexion torques generated by microstimulation in all tested locations of the L6 spinal cord in one animal. The EMG value used in this study is the integration of the rectified raw EMG of the first 12 sec data during each 30 sec of microstimulation. The means of the first 12 sec of isometric torque generated by the 30 sec of microstimulation were used to define the level of extension or flexion torques. The EMG and the torque were generated by microstimulation of 0.2 ms pulsewidth, 40 Hz frequency and 100 μA intensity from 7 microelectrode tracks in the L6 spinal cord. A total of 163 locations were tested in this animal and the location depth ranged from 0.4 mm to 5.4 mm in the L6 spinal cord. As shown in Fig. 3, the extension torques produced by microstimulation in the L6 spinal cord were much larger (about 5 times) than the flexion torque. When extension torque was produced, the extensor EMG was also much larger than the flexor EMG, especially when large extension torque (>50 Ncm) was produced. When flexion torque was produced, the flexor EMG was just slightly larger than the extensor EMG. This means that the co-activation level of the extensors and flexors when extension torque was produced is smaller than the co-activation level when flexion torque was produced. So, the L6 spinal cord is more effective in generating extension torque than flexion torque.

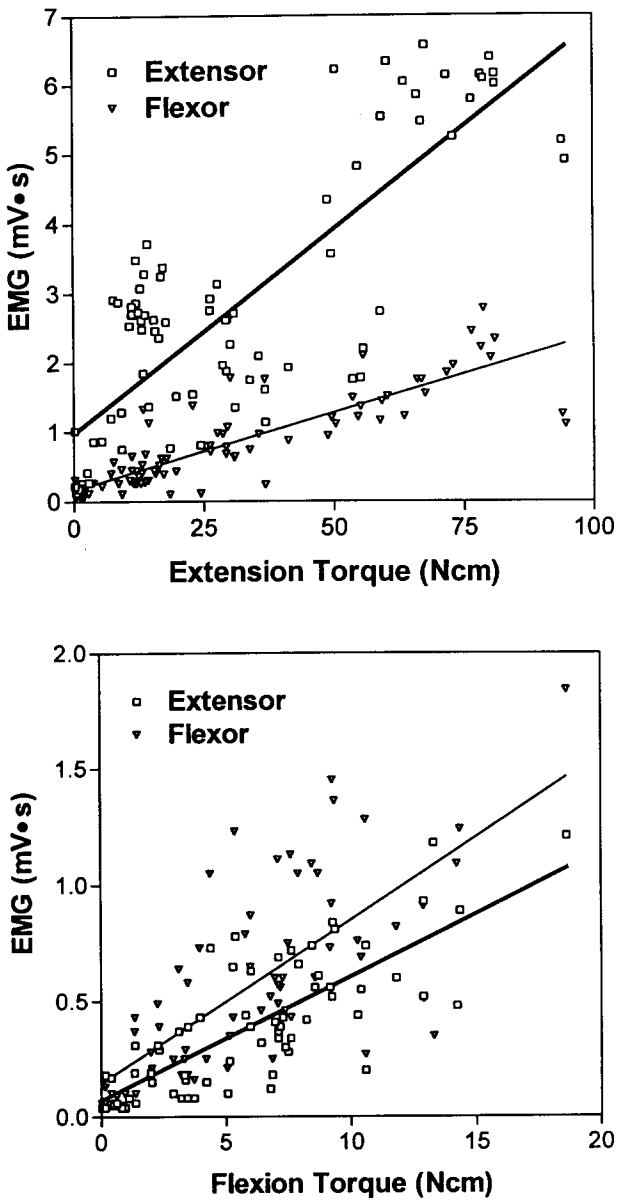

Fig. 3: Relation between the EMG and the isometric extension and flexion torque in all 163 tested locations of the L6 spinal cord. The thick line is the linear regression of the extensor data and the thin line is the linear regression of the flexion data. Note the horizontal and the vertical axis's scale difference between the top and bottom graphs.

IV. DISCUSSION AND CONCLUSION

Using single microelectrode, we studied the knee extensor and flexor EMG activities during microstimulation of the cat L6 spinal cord while monitoring the isometric joint torques. The extensor EMG's were in general larger than the flexor EMG, especially when large extension torque was produced

by microstimulation in the ventral horn of the L6 spinal cord. The flexor EMG was only slightly larger than the extensor EMG and both were small when flexion torque was produced by microstimulation in the dorsal horn of the L6 spinal cord. The extensor EMG increased rapidly with increasing stimulus intensity when extension torque was evoked in the ventral part of the L6 spinal cord, but the flexor EMG did not. These results showed that the L6 spinal cord of the cat is more effective in generating extension torque than flexion torque.

The neuroanatomical studies by Romanes [7] and Vanderhorst et al. [8] found that the motoneurons controlling the knee joint extensor were located in the ventrolateral area of the ventral horn gray matter. Therefore the large extension torques with the large extensor EMG produced in this study are very likely excited by direct activation of the extensor motoneurons and their axons. In contrast, the small flexion torques with the small flexor EMG produced by microstimulations in the dorsal part of the cord is probably due to activation of flexor reflex afferents, rather than to direct electrical excitation of flexor motoneurons.

Our results show that the extensor EMG was large at the same time the flexor EMG was small when a large extension torque was generated by stimulation deep in the L6 ventral horn. This suggests that it be possible to activate extensors and flexors at low co-activation levels by microstimulation at specific sites in the spinal cord. This is important not only to restore functional limb movement but also to control knee joint stiffness for standing. The EMG recordings from the extensors and flexors could only provide a qualitative measurement of the co-activation level of muscle activities, but not a quantitative measurement. The latter can only be done by measuring the forces of the individual extensor and flexor contractions evoked by microstimulation. Such a measurement can not be done in this study because an intact knee joint system was required to study the joint rotation torque produced by microstimulation.

ACKNOWLEDGMENT

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ADDITIVE AND SYNERGISTIC HINDLIMB TORQUE RESPONSES PRODUCED BY FOCAL MICROSTIMULATION OF THE CAT L_6 SPINAL CORD WITH MULTIPLE MICROELECTRODES. J.R. Roppolo*, C. Tai, C. Robinson, W.C. de Groat, & A.M. Booth, Depts. of Pharmacology & Rehabilitation Sci. & Tech., University of Pittsburgh, Pittsburgh, PA 15261

Previous studies from this laboratory have shown that microstimulation of the L_6 spinal cord, using a single fine-tipped microelectrode, could generate large (50-90Ncm) torque responses from the lower hindlimb (shank), about the knee joint. The purpose of the present study was to examine the possibilities of improving these responses by microstimulation with several electrodes, all positioned in the same motor pool and with each electrode providing reduced stimulus current density. Microstimulation with several electrodes also provided the opportunity for examining various stimulus paradigms such as interleaving and simultaneous stimulation as well as the optimal electrode separation. Male cats were anesthetized with pentobarbital (20-25mg/kg iv). A rotational torque sensor was attached to the tibia with its center of rotation over the knee joint. EMG activity from the hind limb flexor and extensor muscle groups was recorded via fine (0.25mm) stainless steel wires placed in the flexor and extensor muscles. A dorsal laminectomy exposed the L_4 to S_3 spinal cord and roots. A fixed array of four fine-tipped (300-400μ^2) activated iridium microelectrodes with either 0.5 or 1 mm electrode separation was used for cord stimulation (10-100μA, 40Hz, 0.2msec pulse width, 30sec on 120sec off). The electrode array was oriented in the rostrocaudal direction parallel with the motoneuron pool of the L_6 spinal cord. The responses elicited at each of the four electrodes were slightly different, although with adjacent electrodes at 0.5mm separation the responses were often similar. Additive responses were generated between two adjacent as well as non-adjacent electrodes with a separation of 3mm (maximum tested). Additive effects were also produced by simultaneous stimulation with 3 & 4 electrodes. Synergistic responses were elicited by 2, 3, or 4 microelectrodes activated simultaneously. The greatest synergism (200-325% greater than additive) was seen when the stimulus intensity was just at or slightly above threshold. Stimulus interleaving with 2, 3, or 4 electrodes decreases the torque response compared to synchronous stimulation. These studies suggest that focal microstimulation with several electrodes can provide enhanced responses at reduced current density. The optimal number of electrodes (maximum of 4 examined in this study) and the ideal distance of separation are still under investigation. (Supported by NIH contract #NO1-5-2332.)

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